Expression and inheritance of nerve insensitivity resistance in larvae of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from China

Jian-Guo Tan and Alan R McCaffery*

School of Animal and Microbial Sciences, The University of Reading, Whiteknights, PO Box 228, Reading RG6 6AJ, UK

Abstract: Nerve insensitivity resistance to synthetic pyrethroids was detected in a resistant field strain (JSFX-R) of the cotton bollworm, *Helicoverpa armigera* (Hübner), using a neurophysiological assay in which extracellular spontaneous neuronal activity was measured in response to *cis*-cypermethrin. The nerve insensitivity mechanism was selected using a combination of toxicological and neurophysiological methods. The third-instar larvae in selected strains of Family-37 and CTR strain expressed a very high resistance to fenvalerate (RF = 2060-fold and 805-fold, respectively) and high cross-resistance to DDT (RF = 1927-fold and 2384-fold, respectively) which was not affected by two metabolic synergists, PBO and DMC. The frequency of nerve-insensitive individuals detected in neurophysiological assays (54, 81 and 100% for JSFX-R strain, and the selected strains Family-43 and Family-37, respectively) was not only positively correlated ($R^2 = 0.968$) with the frequency of non-PBO-synergisable resistant individuals detected in toxicological tests (37.5, 62.5 and 90% for JSFX-R strain, Family-43 and Family-37, respectively), but also positively correlated ($R^2 = 0.978$) with the frequency of DDT-resistant individuals detected in toxicological tests (40, 67.5 and 93.3% for JSFX-R strain, Family-43 and Family-37, respectively). Analysis of dose–mortality lines to DDT and fenvalerate from F_1 hybrids ($R♀ \times S♂$) indicated that nerve insensitivity resistance to DDT and fenvalerate in the CTR strain was inherited in an incompletely recessive pattern. Degree of dominance (D) was estimated to be -0.66 (± 0.06) (DDT) and -0.26 (± 0.04) (fenvalerate). The dose–mortality curves to DDT in backcross progeny were strongly suggested, by chi-square analysis, to be fitted with those expected of a one-gene model. Evidence for the co-existence of nerve insensitivity and oxidative metabolic resistance mechanisms within individual *H armigera* and the effects of their interaction on the expression of resistance to fenvalerate are discussed.

1 INTRODUCTION

The cotton bollworm, *Helicoverpa armigera* (Hübner) (previously *Heliothis armigera*), is the main pest of cotton in China and its control has long been a severe problem for Chinese cotton producers. Pyrethroid insecticides such as fenvalerate and deltamethrin were introduced in 1980 and mainly used in cotton for the control of *H armigera* and the cotton aphid, *Aphis gossypii* (Glov). In 1989 poor control of *H armigera* by fenvalerate was obtained in the major cotton growing areas of Shandong Province and resistance to fenvalerate was subsequently demonstrated by laboratory bioassays.[1]

Resistance to synthetic pyrethroids in *H armigera* has developed in Asia, the Indian subcontinent, Australia and some African countries,[2–8] and is threatening the production of cotton and efficacy of pyrethroid insecticides. The mechanisms of resistance to these compounds have been extensively studied in *H armigera* and include delayed penetration, enhanced metabolic detoxification and reduced nerve sensitivity.[9–15] The *kdr*-like nerve insensitivity resistance mechanism has been shown to be an important mechanism in resistance to pyrethroids and DDT in a number of insect pests, on the basis of cross-resistance patterns and an absence of synergism by metabolic synergists.[16,17] Three possible changes have been thought to account for the reduced sensitivity of nerves to pyrethroids and DDT in *kdr* insects, including structural modifications of the sodium channel leading to reduced receptor binding,[18–20] alterations in the lipid composition of neuronal membranes,[21] and a reduction in the density of sodium channels.[22,23] A number of recent studies have revealed that specific sodium channel mutations may be associated with *kdr*-type nerve insensitivity

* Correspondence to: Alan R McCaffery, Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire RG42 6ET, UK
E-mail: alan.mccaffery@aguk.zeneca.com
Contract/grant sponsor: CVCP
Contract/grant sponsor: Zeneca Agrochemicals
(*Received 16 June 1998; revised version 24 September 1998; accepted 20 January 1999*)

resistance to pyrethroid insecticides.[24–29] A mutation encoding a leucine to phenylalanine change in transmembrane segment 6 of homology domain II (IIS6) appears to be highly conserved in different insect species[24–26] although other mutations may also be involved.[27–29] The existence of this neurophysiologically detectable nerve insensitivity mechanism has been documented in a number of lepidopteran pest species including *Spodoptera littoralis* Boisd,[30] *Trichoplusia ni* (Hübner),[31] *Heliothis virescens* F,[32,33] and *H armigera* from Thailand,[10] Australia[11] and India.[13] The increased frequency of this mechanism in field populations of the insect has resulted from extensive selection with pyrethroids[9,11,34] and has led to severe consequences for its control. Early detection is therefore critical to the development of rational strategies for resistance management.

A thorough knowledge of insecticide resistance genetics and the underlying biochemical and physiological mechanisms is useful for devising strategies of insecticide resistance. The detection, monitoring, modelling and risk assessment of resistance can also greatly benefit from a knowledge of its mode of inheritance. The genetic basis of laboratory-selected resistance to pyrethroids has been studied for only a few species of agricultural insect pests, although Georghiou[35] reported that resistance to pyrethroids has been documented in at least 48 species of insects. With regard to the mode of inheritance of pyrethroid resistance, no consistent pattern has been observed in the lepidopteran pests which have been reported to date. An incompletely recessive pattern was observed for fenvalerate and permethrin resistance in the diamondback moth, *Plutella xylostella* (L),[36,37] and for permethrin resistance in the tobacco budworm.[38] However, an incompletely dominant pattern was obtained for fenvalerate resistance in the cotton bollworm,[39] and for permethrin resistance in the pink bollworm, *Pectinophora gossypiella* (Saunders).[40] This kind of inconsistency could be due to the different mechanisms in the strain observed or their interactions.

We have previously reported that the presence of the nerve insensitivity mechanism, as indicated by neurophysiological study, is involved in fenvalerate resistance in cotton bollworm from China.[41] Herein we report the expression of the nerve insensitivity resistance to DDT and fenvalerate in selected strains of *H armigera* and the inheritance pattern of this mechanism in a selected homozygous (CTR) strain.

2 MATERIALS AND METHODS

2.1 Insects

All the studies were performed using third-instar larvae of various strains of *Helicoverpa armigera*. A laboratory strain, Reading, was used as a susceptible reference strain and had been kept in culture for over 14 years without selection. The JSFX-R strain originated from samples of at least 1000 eggs and neonate larvae of a pyrethroid-resistant population of *H armigera*, collected randomly from cotton plants in the Fengxie county area of Jiangsu province in China in June 1994 and June–July 1995. From this strain, further resistant strains were obtained by selective breeding as described below.

2.2 Insecticides

Technical *cis*-cypermethrin (98.4%) and fenvalerate (97.5%) were supplied by Zeneca Agrochemicals, Jealott's Hill, UK and Shell Research Limited, Sittingbourne, UK, respectively. Piperonyl butoxide (2-(2-butoxyethoxy)ethyl 6-propylpiperonyl ether; PBO) was supplied as technical material (98%) by Endura, Italy. 1,1-bis(4-Chlorophenyl)-2,2,2-trichloroethane (DDT) and 1,1-bis(4-chlorophenyl)-ethanol (DMC) were supplied by Aldrich Chemical Company Ltd, Gillingham, UK, as technical material (98% and 97% respectively).

2.3 Neurophysiological assay for nerve insensitivity

Extracellular spontaneous neuronal activity was recorded in the exposed peripheral nerves of partly dissected third-instar larvae bathed in saline containing *cis*-cypermethrin at 25(±2) °C using a cumulative dose response assay described in detail previously by McCaffery *et al.*[33,41] EC_{50} values were estimated using a probit analysis. Highly resistant insects not responding at 100 nM *cis*-cypermethrin could not be included in this estimation. In Family-37 EC_{50} was estimated as >100 nM because all tested insects responded at or above the highest concentration of 100 nM *cis*-cypermethrin.

2.4 Insecticide and synergist bioassays

The toxicity of fenvalerate and DDT to the third-instar larvae with a body weight of 13–20 mg in all the strains of *H armigera* was determined in full dose mortality topical bioassays as described previously.[4,42] Insecticide was dissolved in analytical grade acetone and appropriate serial dilution were prepared. A fixed volume of insecticide solution (0.2 µl) was topically applied on the thoracic dorsum of each third-instar larva using a Hamilton syringe dispenser. In the case of very high concentrations of DDT, where crystallisation could occur, the solution was diluted and a 1-µl drop of insecticide solution was applied on each larva. Newly moulted larvae or those about to moult were excluded from all tests. Three or four replicates of 10 larvae were treated for the control and each concentration and at least four or five concentrations in the appropriate range were used for each test. All treated insects were kept separately in 30-ml sterile pots with a small block of artificial diet, under the same conditions as the stock insect cultures. Mortality was assessed 72 h after treatment. Larvae were classified as dead if they were unable to change positions within 15 s after being prodded with a blunt seeker. Synergist bioassays were performed by pre-dosing test insects

with 20 μg synergist (PBO or DMC) in 0.2 μl of acetone on the dorsal portion of the abdomen 30 min prior to the application of a range of insecticide doses. Control insects were treated with acetone alone or treated with synergist alone. No mortality was found in control insects treated with acetone or with synergist alone. Toxicity was evaluated using SAS software[43] and Micro-Probit 3.0

2.5 Selection of nerve insensitivity resistance mechanism

In order to form a homozygous strain expressing the nerve insensitivity resistance mechanism, the JSFX-R strain was selected and purified using a combination of single pair mating, toxicological and neurophysiological methods. Insects of the JSFX-R strain from F_2 to F_4 were selected in the third stadium using a diagnostic dose of fenvalerate of 0.04 μg per larva. The offspring of single pair matings at F_5 were screened with a diagnostic dose of fenvalerate following pre-treatment (30 min) with 20 μg PBO. Families which expressed lower mortality to fenvalerate alone, together with lower synergism by PBO, were kept for further selection. Resistant Family-43 at F_{10} was chosen as the line for further selection based on a high resistance to fenvalerate and a low PBO synergistic ratio. Family-37 at F_{14}, which showed high resistance to fenvalerate and which was homozygous for nerve insensitivity, as indicated by the neurophysiological assay, was kept as the nerve-insensitive strain. Between the generations where single pair matings were set up, all third-instar larvae were selected with a diagnostic dose of fenvalerate. Finally, the CTR strain was derived from the offspring of Family-37, which was selected with fenvalerate.

2.6 Inheritance study

The Reading strain was used as the susceptible parent and the CTR strain was used as the resistant parent. Virgin females and males were obtained by determining the sex of pupae. Female and male pupae were held in separate dishes to await adult emergence. Reciprocal F_1 crosses ($S♂ \times R♀$ and $S♀ \times R♂$) were made by mass matings between Reading strain (S) and CTR strain (R). A portion of the F_1 progeny was used in the assessment of dose–motality response. Others were reared to maturity for F_2 crosses which include $F_1♂ \times F_1♀$ and $F_1♂ \times R♀$. The dose–mortality response in all crosses was determined using the topical bioassay methods described above.

2.7 Data analyses

The dose–mortality relationship obtained from F_1 cross and from parents was used to determine the degree of dominance (D value) of fenvalerate and DDT resistance using the formula

$$D = \frac{2X_3 - X_2 - X_1}{X_2 - X_1}$$

where X_1, X_2 and X_3 were the $\log_{10}(LD_{50})$ of susceptible Reading strain, resistant CTR strain and heterozygous F_1 hybrid.[44] The standard error of the dominance value (D) was calculated by taking the square root of variance of D value[45]

$$\mathrm{var}(D) = \frac{4}{(X_2 - X_1)^2} \cdot \left[\mathrm{var}(X_3) + \frac{(X_3 - X_1)^2}{(X_2 - X_1)^2} \cdot \mathrm{var}(X_2) + \frac{(X_3 - X_2)^2}{(X_2 - X_1)^2} \cdot \mathrm{var}(X_1) \right]$$

The standard error (SE) of a D value is useful in determining whether the value of D is significantly different from ±1 or 0. Statistical analysis of back-cross data was carried out with a chi-square test for the goodness-fit between the observed response and expected response at each dose in a series, as described by Preisler *et al.*[45]

$$\chi^2 = \frac{(r_i - n_i p_i)^2}{n_i p_i (1 - p_i)}$$

where n_i = total number of insects from the back-cross generation treated with the ith dose. r_i = observed number of dead at ith dose and p_i = estimated mortality probability under the hypothesised genetic model at ith dose. Expected mortalities were estimated assuming that resistance to DDT or fenvalerate is determined by a single major gene with a Mendelian mode of inheritance.[46] Therefore expected mortality at each dose for the back-cross of $F_1♂ \times R♀$ and inter-cross of $F_1♂ \times F_1♀$ was calculated respectively as:

$$X_i = 0.5 W_{F1} + 0.5 W_{RR}$$
$$X_i = 0.25 W_{SS} + 0.5 W_{F1} + 0.25 W_{RR}$$

where W_{F1} is the observed mortality at ith dose of the F_1 hybrid ($S♂ \times R♀$), W_{RR} is the observed mortality at ith dose of resistant homozygous parent CTR strain, and W_{SS} is observed mortality at ith dose of susceptible homozygous parent Reading strain. The genetic hypothesis was then tested by comparing the test static χ_i^2 (for each $i = 1 \ldots N$) with values from χ^2 table with 1 df, or by comparing the sum $\chi^2 = \sum_i^N \chi_i^2$ with values from χ^2 table with N df.

3 RESULTS

3.1 Resistance to fenvalerate and DDT and synergism

3.1.1 Resistance to fenvalerate

A full dose–mortality assay with fenvalerate was conducted using the Reading susceptible strain, the JSFX-R field strain, and the selected CTR strain. A high level of resistance to fenvalerate (RF = 200-fold at the LD_{50} value) was observed in the JSFX-R strain relative to the Reading strain. After selection, the resistance to fenvalerate increased to 2060-fold in Family-37 and 805-fold in the CTR strain (Table 1). Pre-treatment of the Reading susceptible strain with

Strain	Treatment	LD_{50} (μg per larva) (95% fiducial limits)	RF[a]	SR[b]	Slope (±SE)
Reading	Fenvalerate	0.003 (0.0027∼0.0033)	1.0		2.9 (±0.2)
Reading	Fenvalerate+PBO	0.008 (0.0063∼0.0095)	2.7	0.4	2.4 (±0.3)
JSFX-R	Fenvalerate	0.60 (0.40∼1.09)	200		1.6 (±0.3)
JSFX-R	Fenvalerate+PBO	0.018 (0.013∼0.027)	6.0	33.5	1.4 (±0.2)
Family-37	Fenvalerate	6.18 (4.17∼8.91)	2060		1.1 (±0.1)
Family-37	Fenvalerate+PBO	0.14 (0.11∼0.18)	46.7	44.1	1.8 (±0.2)
CTR	Fenvalerate	1.69 (1.25∼2.53)	805		1.3 (±0.1)

Table 1. Toxicity of fenvalerate to third-instar larvae of the Reading susceptible and various resistant strains of *Helicoverpa armigera* and synergism with piperonyl butoxide (PBO)

[a] Resistance factor (RF) expressed as LD_{50} of resistant strain/LD_{50} of Reading strain.
[b] Synergistic ratio (SR) expressed as LD_{50} without PBO/LD_{50} with PBO.

	Reading strain			Family-37			
Treatment	LD_{50} (μg per larva)	Slope (±SE)	SR[b]	LD_{50} (μg per larva)	Slope (±SE)	SR[b]	RF[a]
DDT	0.26	2.16 (±0.41)		501	0.93 (±0.18)		1927
DDT+PBO	0.43	2.94 (±0.47)	0.60	558	0.92 (±0.20)	0.90	1298
DDT+DMC	0.39	2.04 (±0.40)	0.67	548	0.72 (±0.19)	0.91	1405

Table 2. Toxicity of DDT to third-instar larvae of susceptible Reading strain and resistant Family-37 of *Helicoverpa armigera* and synergism with PBO and DMC

[a] Resistance factor (RF) expressed as LD_{50} of resistant Family-37/LD_{50} of Reading strain.
[b] Synergistic ratio (SR) expressed as LD_{50} without synergist/LD_{50} with synergist.

Table 3. The nerve insensitivity level in different strains, and their toxicological response to fenvalerate and DDT

	Neurophysiology		Toxicology		
Strain	Nerve insensitivity (%) (n)	EC_{50} (nM) (95% fiducial limits)	Resistance to fenvalerate (%)	Resistance to fenvalerate/PBO (%)	Resistance to DDT (%)
Reading	0 (44)	1.2 (0.7∼1.8)	0	0	0
JSFX-R	54 (26)	12.7 (7.9∼20.6)	97.5	37.5	40.0
Family-43	81 (26)	39.9 (26.7∼59.8)	73.3	62.5	67.5
Family-37	100 (49)	>100	100	90.0	93.3

Nerve-insensitive individuals were those responding to *cis*-cypermethrin at or above a concentration of 100 nM in the neurophysiological assay. Reading strain individuals all responded at or below 50 nM *cis*-cypermethrin. The values in toxicological tests represent the percentage of resistant individuals surviving the discriminating dose of fenvalerate (0.04 μg perlarva), fenvalerate/PBO (0.04/20 μg) and DDT (8 μg per larva).

PBO resulted in no synergism of fenvalerate, but pre-treatment of the JSFX-R strain and Family-37 gave 33.5-fold and 44.1-fold synergism to fenvalerate, respectively (Table 1). The non-PBO-synergisable resistance increased from 6.0-fold in the JSFX-R strain to 46.7-fold in Family-37. This suggested that the nerve insensitivity resistance mechanism was preferentially selected in this process.

3.1.2 Cross-resistance to DDT

A very strong cross-resistance to DDT was observed in larvae from the fenvalerate-resistant Family-37 and CTR strain. The resistance to DDT was over 1927-fold and 2384-fold (at the LD_{50} value) relative to the Reading susceptible strain (see Tables 2 and 4). This resistance was not suppressed by pre-treatment of larvae with DMC (a DDT dehydrochlorinase inhibitor) or PBO (a monooxygenase inhibitor) (Table 2), providing further indirect evidence for the presence of nerve insensitivity resistance in these strains.

3.1.3 Correlation between neurophysiological and toxicological responses

The results shown in Table 3 and Fig 1a illustrate that there was a significant positive correlation ($R^2 = 0.968$) between the frequency of nerve-insensitive individuals, detected in neurophysiological assays, and the frequency of non-PBO-synergisable individuals detected in toxicological tests. In the JSFX-R strain the non-PBO-synergisable individuals comprised 37.5% of the population, while in Family-43 and Family-37 the non-PBO-synergisable individuals comprised 67.5 and 90% of the population, respectively (Table 3). The presence of the nerve insensitivity resistance mechanism was again demonstrated by the positive correlation ($R^2 = 0.978$) between the frequency of DDT-resistant individuals

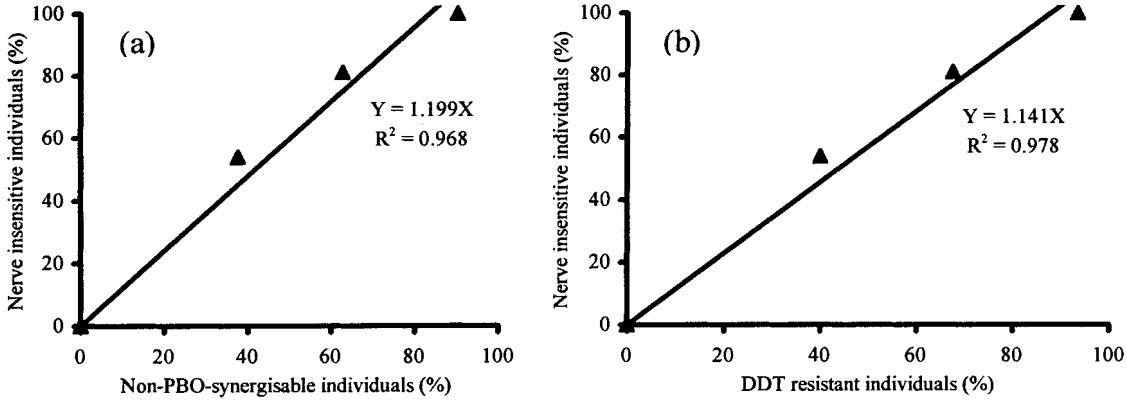

Figure 1. Correlation between (a) non-PBO-synergisable individuals detected in toxicological tests and nerve-insensitive individuals detected in neurophysiological assays and (b) DDT-resistant individuals detected in toxicological tests and nerve-insensitive individuals detected in neurophysiological assays. Significance level $P < 0.05$.

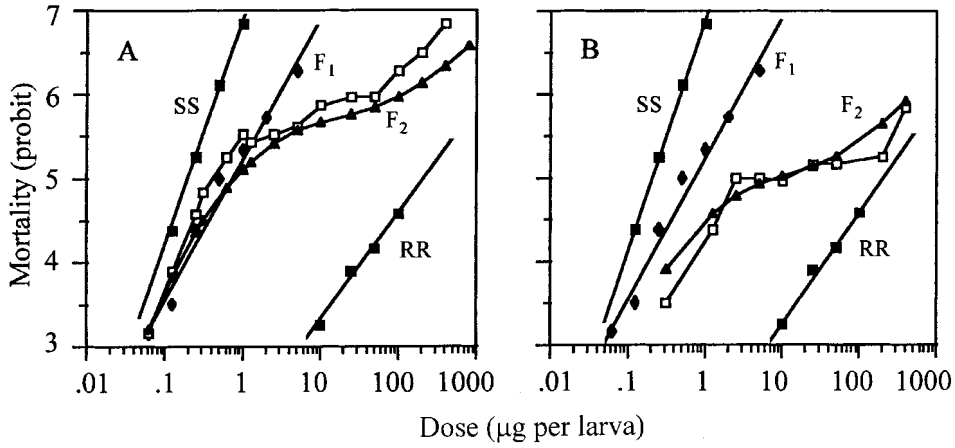

Figure 2. Dose–mortality responses for genetic crosses of nerve insensitivity resistant CTR strain of *Helicoverpa armigera* when tested with DDT. The solid line (▲) shows the expected response for (A) a 1:2:1 segregation in the inter-cross of $F_1♂ \times F_1♀$ and for (B) a 1:1 segregation in the backcross of $F_1♂ \times R♀$. The expected mortalities at each dose are estimates based on simple Mendelian inheritance of one-locus model (Georghiou, Reference 46).

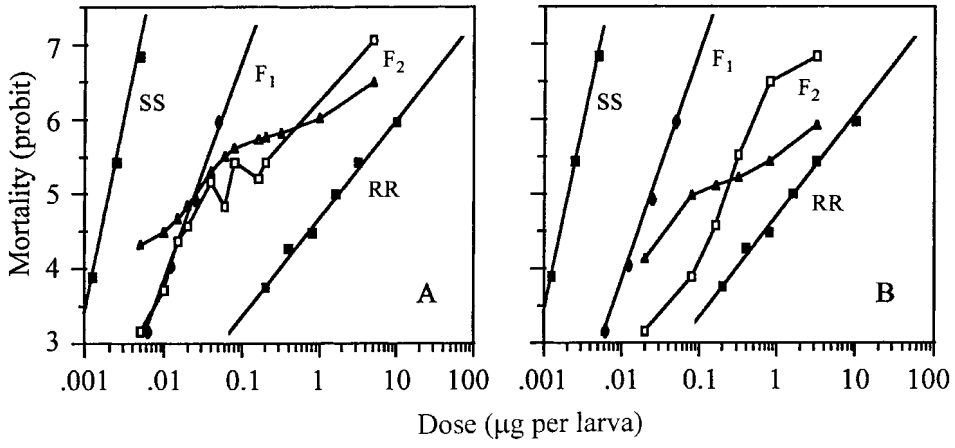

Figure 3. Dose–mortality responses for genetic crosses of nerve insensitivity resistant CTR strain of *Helicoverpa armigera* when tested with fenvalerate. The solid line (▲) shows the expected response for (A) a 1:2:1 segregation in the inter-cross of $F_1♂ \times F_1♀$ and for (B) a 1:1 segregation in the back-cross of $F_1♂ \times R♀$. The expected mortalities at each dose are estimated based on simple Mendelian inheritance of one-locus model (Georghiou, Reference 46).

shown by the percentage of insects surviving the twice discriminating dose of DDT (8μg per larva) in toxicological tests and the frequency of nerve-insensitive individuals in neurophysiological assays (Fig 1b). In the JSFX-R strain the DDT-resistant individuals comprised 40% of the population, and this value increased to 67.5% in Family-43 and 93.3% in Family-37 (Table 3).

Strain or cross	n	LD_{50} (μg per larva) (95% fiducial limits)	RF[a]	Slope (±SE)	D (±SE)[b]
Fenvalerate					
Reading (SS)	120	0.0021 (0.0018∼0.0024)		4.96 (±0.87)	
CTR (RR)	460	1.69 (1.25∼2.53)	805	1.34 (±0.13)	
F_1 (S♂ × R♀)	120	0.067 (0.050∼0.094)	31.9	3.14 (±0.51)	−0.26 (±0.04)
DDT					
Reading (SS)	120	0.21 (0.15∼0.26)		2.80 (±0.50)	
CTR (RR)	200	501 (253∼1912)	2384	0.93 (±0.18)	
F_1 (S♂ × R♀)	210	0.67 (0.51∼0.91)	3.2	1.72 (±0.20)	−0.66 (±0.06)

Table 4. Responses to fenvalerate and DDT of the third-instar larvae of *Helicoverpa armigera* from the homozygous susceptible Reading strain (SS) and resistant CTR strain (RR), and the F_1 (SS♂ × RR♀)

[a] Resistance factor (RF) was determined by dividing the LD_{50} of resistant strain by the LD_{50} of susceptible Reading strain.

[b] The value of dominance D = −1, completely recessive; D = 0, intermediate or co-dominant; D = +1, completely dominant.

3.2 Inheritance

3.2.1 F_1 dose–response

Responses to DDT and fenvalerate of the susceptible Reading strain, the resistant CTR strain and their crosses are given in Table 4 and Figs 2 and 3. Resistances to DDT and fenvalerate were 2384-fold and 805-fold, respectively, in the CTR strain and 3.2-fold and 11.9-fold, respectively, in the progenies of cross (S♂ × R♀). Although the maternal influence on the resistance cannot be evaluated, the degree of dominance and its standard error were −0.66 (±0.06) for DDT resistance and −0.26 (±0.04) for fenvalerate resistance. These results clearly indicated that resistance to DDT and to fenvalerate in the CTR strain was inherited in an incompletely recessive manner.

3.2.2 F_2 dose–response

The dose–mortality curves to DDT displayed an obvious plateau at 50% mortality in the offspring of F_1 back-cross to resistant parent CTR strain, and a plateau at 75% mortality in the progenies of F_1 inter-cross (Fig 2). Chi-square analysis indicated that the observed dose–mortality curves to DDT in F_2 of F_1♂ × R♀ ($\chi^2_{(0.05)[14]} = 13.08$) and F_1♂ × F_1♀ ($\chi^2_{(0.05)[9]} = 7.94$) were respectively fitted to the expected curves calculated from a monogenic model (Fig 2). Together with the lack of synergism by PBO and DMC in toxicological tests and the observed neurophysiological response, these results showed clearly that resistance to DDT in CTR strain is entirely due to the presence of a mechanism of nerve insensitivity. In contrast, the dose–mortality curves to fenvalerate, in F_2 progenies (Fig 3) were different from the curves expected of a one-gene model. It therefore appears that more than one mechanism was involved in the resistance to fenvalerate, as illustrated by the expression of PBO synergism to fenvalerate in the strain (Table 1).

4 DISCUSSION

The results presented here strongly suggest that a nerve insensitivity resistance mechanism plays an important role in the resistance to fenvalerate and DDT in the JSFX-R strain of the cotton bollworm collected from China and in the strains derived from the JSFX-R strain. Individuals expressing this mechanism were selected in these studies and, as a result, a strain which was homozygous for the mechanism was obtained. Previous neurophysiological work has concluded that nerve insensitivity is an important mechanism of resistance to pyrethroids in Thai and Indian strains of *H armigera*.[10,13] In a study of nerve insensitivity in *H armigera* in Australia, the time to burst discharge to a high dose of fenvalerate (10^{-5}M) was significantly different between resistant and susceptible insects and could be used to classify the resistant and susceptible insects.[11] Together these studies have demonstrated the widespread nature of a nerve insensitivity resistance mechanism which confers resistance to pyrethroid insecticides in *H armigera*.[41]

The expression of this nerve insensitivity resistance mechanism usually confers cross-resistance to DDT and pyrethroids, despite their different chemical structures,[47] because DDT and pyrethroids are known to exert their toxic effects by binding to and modifying the normal function of voltage-sensitive sodium channels in a very similar way.[48,49] The appearance of cross-resistance to DDT in a strain resistant to pyrethroids has, therefore, very often been taken as an indication that the nerve insensitivity mechanism is involved in the resistance. Our results (Table 2) indicated that in Family-37 and the CTR strain, which was shown to be homozygous for nerve insensitivity, there is a high level of cross-resistance to DDT without any synergistic action by PBO and DMC. The phenomenon of cross-resistance between pyrethroids and DDT has been well documented in other lepidopteran species such as *Heliothis virescens*,[50,51] *Phyllonorycter blancardella* F,[52] *Plutella xylostella*,[53] and has been generally thought to be due to the existence of a nerve insensitivity resistance mechanism.[17,54] The positive correlation ($R^2 = 0.978$) between the percentages of DDT-resistant individuals and nerve-insensitive individuals (Fig 1b) provides

more reliable evidence in support of the presence of cross-resistance caused by the nerve insensitivity mechanism.

Futher evidence for the involvement of the nerve insensitivity mechanism is the absence of synergism of pyrethroids or DDT by metabolic synergists such as PBO, DEF (*S,S,S*-tributyl phosphorotrithioate), and DMC.[55] Liu *et al*[53] found resistance to DDT and pyrethroids was unaffected by metabolic synergists in the diamondback moth, *P xylostella*, suggesting that a *kdr* factor could be a resistance mechanism. In studies on mechanisms of resistance to pyrethroids in *H virescens*, non-PBO-synergisable resistance was thought to be due to the nerve insensitivity mechanism.[56] A significant correlation was found between the numbers of non-PBO-synergisable resistant individuals and the numbers of nerve-insensitive individuals in *H virescens*.[33] The results presented here also indicate a positive correlation between the frequency of nerve-insensitive individuals and non-PBO-synergisable individuals in the fenvalerate-resistant JSFX-R strain and selected strains of *H armigera* ($R^2 = 0.968$). The frequency of non-PBO-synergisable individuals or the frequency of DDT-resistant individuals detected in a resistant field population of *H armigera* could therefore indicate the importance of nerve insensitivity resistance mechanism in a population.

Although Family-37 was confirmed by neurophysiological experiments to be a strain homozygous for nerve insensitivity, the resistance to fenvalerate could still be suppressed to a moderate degree by PBO (Table 1). The contribution of each resistance mechanism to the overall fenvalerate resistance (2060-fold) was 44.1-fold (48.6%) for the non-PBO-synergisable factor, presumed to be largely the nerve insensitivity resistance mechanism, and 46.7-fold (51.4%) for the PBO-synergisable resistance mechanism, if interaction of the two resistance factors in combination was multiplicative.[57] These results imply that the oxidative metabolic resistance mechanism can coexist with the nerve insensitivity resistance mechanism in individual larvae, and could explain the interaction of these two resistance mechanisms on the expression of resistance to pyrethroids. In a field population of *H armigera* in Australia, pyrethroid selection pressure in the field was found clearly to select for the oxidative metabolic resistance mechanism.[7] Similarly, in this laboratory, mass selection of third-instar larvae of the JSFX-R strain with fenvalerate by topical application led to a dramatic increase in the level of fenvalerate resistance which could be suppressed by PBO (data not shown). This is likely to be due to the differential genetic dominance of each resistance mechanism. The oxidative metabolic resistance mechanism in Australian *H armigera* has been shown to be semi-dominant,[39] whilst the nerve insensitivity resistance mechanism has been considered to be recessive in other insects.[54,58] The obviously different fitness costs associated with these two resistance mechanisms may have also contributed to the different selection.[59] In field populations it is possible that the oxidative metabolic resistance mechanism may protect the nerve insensitivity resistance mechanism, when these two mechanisms coexist in the same individual.

The genetic studies showed that resistance to DDT and fenvalerate in the CTR strain was inherited in an incompletely recessive manner and demonstrated a general characteristic of the nerve insensitivity mechanism.[54,58] The dose–mortality response to DDT in the back-crosses (Fig 2), relative to the expected dose–response based on a one-gene model, strongly suggested that nerve insensitivity was the major mechanism conferring resistance to DDT in the CTR strain, whilst the dose–mortality response to fenvalerate in the back-crosses (Fig 3) indicated more than one mechanism contributed to resistance to fenvalerate in the CTR strain. Interestingly, the differential of dose–mortality response to DDT and to fenvalerate in the F_2 progeny suggested an alternative way to study the genetics of this specific resistance mechanism in a strain, although there might be more than one mechanism included. Because DDT resistance in the CTR strain was not affected by metabolic synergists such as PBO and DMC (Table 2), the dose–mortality response to DDT could be used as the phenotypic expression of nerve insensitivity in a study of the genetics of this mechanism. Resistance to fenvalerate in the CTR strain involved the co-existence of nerve insensitivity and oxidative metabolism. To test for linkage and the possible interaction of the nerve insensitivity gene with oxidative metabolism, the two factors must first be isolated in isogenic strains. This is presently being undertaken in this laboratory using a series of crosses and back-crosses, together with single-pair mating.

The main aim of insecticide resistance management (IRM) is to conserve or recover susceptibility to an insecticide in a pest population using modified insecticide use patterns which reduce selection.[60] The results of this study clearly showed that the substantial resistance to fenvalerate in the field resistant strain (JSFX) of *H armigera* from China could be eliminated by low doses of fenvalerate following PBO pre-treatment. This implies that metabolic detoxification, probably via mono-oxygenases, was the major mechanism of resistence in this population. In the field, therefore, a mixture of pyrethroid plus PBO could be used as a strategy for resistance management and to retain susceptibility to the pyrethroids. Although selection with a mixture of a pyrethroid and PBO could lead to the development of a nerve insensitive resistance mechanism, this should be more easily managed due to its genetically recessive nature and associated higher fitness costs. Nevertheless, care would need to be taken to avoid the emergence of a *super-kdr* type of nerve insensitivity resistance mechanism with metabolic resistance mechanisms which would lead to the development of a highly resistant population.[61,62]

The identification of nerve insensitive and metabolic

resistance to pyrethroids in Chinese strains of *H armigera* leads to more fundamental considerations of the design of management strategy to control resistance. Resistance to endosulfan, OPs and carbamates arises through mechanisms usually entirely distinct from these identified here in resistance to pyrethroids. A management strategy which employs rotation with endosulfan, OPs and certain carbamates could thus prove useful in reducing the frequency of pyrethroid resistance genes in field populations. Likewise, the use of unsprayed refugia might also prove of enormous benefit to the survival of susceptible alleles and we believe that consideration of these approaches is now appropriate.

ACKNOWLEDGEMENTS

The present research is part of a Ph D program of the first author, and sincere thanks go to Nanjing Agricultural University for valuable assistance during the program, the CVCP for provision of an Overseas Research Studentship Award to Jian-Guo Tan and Zeneca Agrochemicals for financial support. We thank Dr Neil Oldham for valuable suggestions and review of an early draft of this manuscript. We also thank Dr Robert M Perrin for his help in obtaining insects from China. This work was carried out under MAFF Licence Number PHL 1/1973(7/96) issued under the Plant Health (Great Britain) Order 1993.

REFERENCES

1 Shen JL, Wu YD, Tan JG and Tan FJ, Pyrethroid resistance in *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae) in China. *Resistant Pest Management* **4**:22–24 (1992).

2 Gunning RV, Easton CS, Greenup LR and Edge VE, Pyrethroid resistance in *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae) in Australia. *J Econ Entomol* **77**:1283–1287 (1984).

3 Ahmad M and McCaffery AR, Resistance to insecticides in a Thailand strain of Heliothis armigera (Hübner) (Lepidoptera: Noctuidae) *J Econ Entomol* **81**:45–48 (1988).

4 McCaffery AR, King ABS, Walker AJ and El-Nayir H, Resistance to synthetic pyrethroids in the bollworm *Heliothis armigera*, from Andhra Pradesh, India. *Pestic Sci* **27**:65–66 (1989).

5 McCaffery AR, Walker AJ and Topper CP, Insecticide resistance in the bollworm, *Helicoverpa armigera* from Indonesia. *Pestic Sci* **32**:85–90 (1991).

6 McCaffery AR, Resistance to insecticides in Heliothine Lepidoptera: a global view. *Phil Trans. R Soc Lond B* **353**:1–16 (1998).

7 Forrester NW, Cahill M, Bird LJ and Layland JK, Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia. *Bull Entomol Research Suppl* **1**:1–132 (1993).

8 Ahmad M, Arif MI and Attique MR, Pyrethroid resistance of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. *Bull Entomol Res* **87**:343–348 (1997).

9 McCaffery AR, Mechanisms of resistance to pyrethroids in *Heliothis* and *Helicoverpa* species, in *1994 Proc Beltwide Cotton Conf*, vol **2**, National Cotton Council, Memphis, TN, USA. pp 836–837 (1994).

10 Ahmad M, Gladwell RT and McCaffery AR, Decreased nerve insensitivity is a mechanism of resistance in a pyrethroid-resistant strain of *Helicoverpa armigera* from Thailand. *Pestic Biochem Physiol* **35**:165–171 (1989).

11 Gunning RV, Easton CS, Balfe ME and Ferris IG, Pyrethroid resistance mechanisms in Australia *Helicoverpa armigera*. *Pestic Sci* **33**:473–490 (1991).

12 Ahmad M and McCaffery AR, Elucidation of detoxication mechanisms involved in resistance to insecticides in the third-instar larvae of a field-selected strain of *Helicoverpa armigera* with the use of synergists. *Pestic Biochem Physiol* **41**:41–45 (1991).

13 West AJ and McCaffery AR, Evidence for nerve insensitivity to cypermethrin from Indian strains of *Helicoverpa armigera* in *Proc Brighton Crop Protec Conf*. pp 233–238 (1992).

14 Gunning RV, Moores GD and Devonshire AL, Esterases and esfenvalerate resistance in Australian *Helicoverpa armigera* (Hübner) Lepidoptera: Noctuidae. *Pestic Biochem Physiol.* **54**:12–23 (1996).

15 Kranthi KR, Armes NJ, Rao NGV, Raj S and Sundaramurthy VT, Seasonal dynamics of metabolic mechanisms mediating pyrethroid resistance in *Helicoverpa armigera* in central India. *Pestic Sci* **50**:91–98 (1997).

16 Osborne MP and Pepper DR, Mechanisms of *kdr* and super-*kdr* resistance, in *Molecular Mechanisms of Insecticide Resistance Diversity Among Insects*, ed by Mullin CA and Scott JG, American Chemical Society, Washington DC. 71–89 (1992).

17 Soderlund DM, Molecular mechanisms of insecticide resistance, in *Molecular Mechanisms of Resistance to Agrochemicals*, ed by Sjut V, Springer–Verlag, Berlin. pp 21–56 (1997).

18 Salgado VL, Irving SN and Miller TA, Depolarisation of motor nerve terminals by pyrethroids in susceptible and *kdr*-resistant house flies. *Pestic Biochem Physiol* **20**:100–114 (1983).

19 Pauron D, Barhanin J, Amichot M, Pralavorio M, Berge J-B and Lazdunski M, Pyrethroid receptor in the insect Na^+ channel: alteration of its properties in pyrethroid-resistant flies. *Biochemistry* **28**:1673–1677 (1989).

20 Church CJ and Knowles CO, Relationship between pyrethroid enhanced batrachotoxinin A 20-α-benzoate binding and pyrethroid toxicity to susceptible and resistant tobacco budworm moths *Heliothis virescens*. *Comp Biochem Physiol* **104C**:279–287 (1993).

21 Chiang C and Devonshire AL, Changes in membrane phospholipids, identified by Arrhenius plots of acetylcholinesterase and associated with pyrethroid resistance (*kdr*) in houseflies (*Musca domestica*). *Pestic Sci* **13**:156–160 (1982).

22 Chang CP and Plapp FW Jr, DDT and pyrethroid: receptor binding in relation to knockdown resistance (*kdr*) in the housefly. *Pestic Biochem Physiol* **20**:86–91 (1983).

23 Rossignol DP, Reduction in number of nerve membrane sodium channels in pyrethroid-resistant house flies. *Pestic. Biochem. Physiol* **32**:146–152 (1988).

24 Williamson MS, Martinez-Torres D, Hick CA and Devonshire AL, Identification of mutations in the housefly *para*-type sodium channel gene associated with knockdown resistance (*kdr*) to pyrethroid insecticides. *Mol Gen Genetics* **252**:51–60 (1996).

25 Williamson MS, Martinez-Torres D, Hick CA, Castells N and Devonshire A, Analysis of sodium channel gene sequences in pyrethroid-resistant houseflies in *Molecular Genetics and Evolution of Pesticide Resistance* ed Brown TM, American Chemical Society, Washington DC. pp52–61 (1996).

26 Dong K, A single amino acid change in the *para*-sodium protein is associated with knockdown-resistance (*kdr*) to pyrethroid insecticides in German Cockroach. *Insect Biochem Mol Biol* **27**:93–100 (1977).

27 Park Y and Taylor MFJ, A novel mutation L1029H in sodium channel gene *hscp* associated with pyrethroid resistance for *Heliothis virescens* (Lepidoptera: Noctuidae). *Insect Biochem Mol Biol* **27**:9–13 (1997).

28 Head DJ, McCaffery AR and Callaghan A, Novel mutations in the *para*-homologous sodium channel gene associated with phenotypic expression of nerve insensitivity resistance to

pyrethroids in heliothine lepidoptera. *Insect Mol Biol* **7**:1–6 (1998).
29 Park Y, Taylor MFJ and Feyereisen R, A valine 421 to methionine mutation in IS6 of the *hscp* voltage-gated sodium channel associated with pyrethroid resistance in *Heliothis virescens*. *Biochem Biophy Res Comm* **239**:688–691 (1997).
30 Gammon DW, Pyrethroid resistance in a strain of *Spodoptera littoralis* is correlated with decreased sensitivity of the CNS *in vitro*. *Pestic Biochem Physiol* **13**:53–62 (1980).
31 Salgado VL, Irving SN and Miller TA, The importance of nerve terminal depolarization in pyrethroid poisoning of insects. *Pestic Biochem Physiol* **20**:169–182 (1983).
32 Nicholson RA and Miller TA, Multifactorial resistance to *trans*-permethrin in field-collected strains of the tobacco budworm *Heliothis virescens* F. *Pestic Sci* **16**:561–570 (1985).
33 McCaffery AR, Holloway JW and Gladwell RT, Nerve insensitive resistance to cypermethrin in larvae of the tobacco budworm, *Heliothis virescens*, from USA cotton field populations. *Pestic Sci* **44**:237–247 (1995).
34 Gunning RV, Forrester NW, Easton CS and Greenup LR, Relationship between DDT and Pyrethroid resistance in *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae) in Australia. *Trop Pest Man* **36**:293–295 (1990).
35 Georghiou GP, Overview of insecticide resistance, in *Managing Resistance to Agrochemicals from Fundamental Research to Practical Strategies*, ed by Green MB, LeBaron HM and Moberg WK, American Chemical Society, Washington, DC. pp 18–41 (1990).
36 Liu MY, Tzeng YJ and Sun CN, Diamondback moth resistance to several synthetic pyrethroids. *J Econ Entomol* **74**:393–396 (1981).
37 Yu SJ, Inheritance of insecticide resistance and microsomal oxidases in the diamondback moth (Lepidoptera: Yponomeutidae). *J Econ Entomol* **86**:680–683 (1993).
38 Payne GT, Blenk RG and Brown TM, Inheritance of permethrin resistance in the tobacco budworm (Lepidoptera: Noctuidae). *J Econ Entomol* **81**:65–73 (1988).
39 Daly JC and Fisk JH, Inheritance of metabolic resistance to the synthetic pyrethroids in Australian *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Bull Entomol Res* **82**:5–12 (1992).
40 Osman AA, Watson TF and Sivasupramaniam S, Inheritance of permethrin resistance in the pink bollworm (Lepidoptera: Gelechiidae). *J Econ Entomol* **85**:335–339 (1992).
41 McCaffery AR, Head DJ, Tan J, Dubbeldam AA, Subramaniam VR and Callaghan A, Nerve insensitivity resistance to pyrethroids in Heliothine Lepidoptera. *Pestic Sci* **51**:315–320 (1997).
42 Tan J, Tan F and You Z, Monitoring and selection of resistance in cotton bollworm, *Heliothis armigera*, to four pyrethroids. *J Nanjing Agric University* **4**:36–43 (1987).
43 SAS Institute, *SAS Users Guide: Statistics*, 5th edn, SAS Institute, Cary, NC (1988).
44 Stone BF, A formula for determining degree of dominance in cases of monofactorial inheritance of resistance to chemicals. *Bull WHO* **38**:325–326 (1968).
45 Preisler HK, Hoy MA and Robertson JL, Statistical analysis of modes of inheritance for pesticide resistance. *J Econ Entomol* **83**:1649–1655 (1990).
46 Georghiou GP, Genetics of resistance to insecticides in houseflies and mosquitoes. *Exp Parasit* **26**:224–255 (1969).
47 Bloomquist JR, Neurophysiological assays for the characterisation and monitoring of pyrethroid resistance. *Neurotox'* **88**:543–551 (1988).
48 Soderlund DM and Bloomquist JR, Neurotoxic actions of pyrethroid insecticides. *Ann Rev Entomol* **34**:77–96 (1989).
49 Miller TA and Salgado VL, The mode of action of pyrethroids on insects, in *The Pyrethroid Insecticides* ed by Leahey JP, Taylor & Francis, London and Philadelphia. pp43–97 (1985).
50 Brown MA, Temperature-dependent pyrethroid resistance in a pyrethroid-selected colony of *Heliothis virescens* F (Lepidoptera: Noctuidae). *J. Econ Entomol* **80**:330–337 (1987).
51 Brown TM, Bryson PK and Payne GT, Synergism by propynyl aryl ethers in permethrin-resistant tobacco budworm larvae, *Heliothis virescens*. *Pestic Sci* **46**:323–327 (1996).
52 Pree DJ, Marshall DB and Archibald DE, Resistance to pyrethroid insecticides in the spotted tentiform leafminer, *Phyllonorycter blancardella*, in southern Ontario Canada. *J Econ Entomol* **79**:318–322 (1986).
53 Liu MY, Sun CN and Huang SW, Absence of synergism of DDT by piperonyl butoxide and DMC in larvae of the diamondback moth (Lepidoptera: Yponomentidae). *J Econ Entomol* **75**:964–965 (1982).
54 Sawicki RM, Resistance to pyrethroid insecticides in arthropods, in *Progress in Pesticide Biochemistry and Toxicology*, Vol **5**, Insecticides ed by Hutson DH and Roberts TR, John Wiley & Sons Ltd, New York. pp143–193 (1985).
55 Scott JG, Investigating mechanisms of insecticide resistance: methods, strategies and pitfalls in *Pesticide Resistance in Arthropods* ed by Roush RT and Tabashink BE, Chapman and Hall, London. pp 237–260 (1990).
56 Ottea JA, Ibrahim SA, Younis AM, Young RJ, Leonard BR and McCaffery AR, Biochemical and physiological mechanisms of pyrethroid resistance in *Heliothis virescens* (F). *Pestic Biochem Physiol* **51**:117–128 (1995).
57 Plapp FW Jr, On the molecular biology of insecticide resistance, in *Biochemical Toxicology of Insecticides* ed by O'Brien RD and Yamamoto I, Academic Press, New York, London. pp 179–192 (1970).
58 Plapp FW, Genetics and biochemistry of insecticide resistance in arthropods: prospects for the future, in *Pesticide Resistance: Strategies and Tactics for Management* ed. by National Research Council, Washington DC, National Academy Press. 74–86 (1986).
59 Dubbeldam A and McCaffery AR, Fitness costs associated with pyrethroid resistance in *Heliothis virescens*. *Proc Brighton Crop Prot Conf–Pests and Diseases*. pp 429–430 (1996).
60 Denholm I and Rowland MW, Tactics for managing pesticide resistance in arthropods: theory and practice. *Ann Rev Entomol* **81**:91–112 (1992).
61 Farnham AW, Murray AWA, Sawicki RM, Denholm I and White JC, Characterisation of the structure–activity relationship of *kdr* and two variants of super-*kdr* to pyrethroids in the housefly (*Musca domestica* L). *Pestic Sci* **19**:209–220 (1987).
62 Sawicki RM, Farnham AW, Denholm I and Church VJ, Potentiation of super-*kdr* resistance to deltamethrin and other pyrethroids by an intensifier (factor 161) on autosome 2 in the housefly *(Musca domestica* L). *Pestic Sci* **17**:483–488 (1986).